**SUPPLEMENTARY INFORMATION**

**Low-noise Plasmonic Nanopore Biosensors for Single Molecule Detection at Elevated Temperatures**

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**Experimental**

***Device Fabrication:***

Py-SiNx devices were fabricated as follows. Pyrex substrates (10 mm × 10 mm, 200 μm thick) were coated on both sides with amorphous Si (a-Si, 200 nm thick), using low pressure chemical vapour deposition. Photolithography and reactive ion etching (RIE) were used to “open” a 5 μm × 5 μm window in the a-Si on the topside of the wafer, before a HF wet etch (49 wt%, 5 min) of the exposed Pyrex. Photolithography and RIE were again used on the a-Si layer on the bottom side of the wafer, to define a 100 μm × 100 μm opening, after which a HF wet etch (49 wt%) of the exposed Pyrex was used to merge the two etched chambers (Figure 1B). SiNx layer were prepared separately via low-stress LPCVD deposition onto a 500 μm thick Si substrate. After photolithography and reactive ion etching, KOH wet etch was used to form freestanding 100nm thick SiNx membrane (2 mm × 2 mm window) on a 10 mm × 10mm Si chip. The SiNx membrane was transferred on to the topside of the Pyrex substrate in D.I. water environment. After drying, 20nm thick SiNx membrane was formed by CF4 plasma etching (etch rate of ~15nm/min). Finally, a 50 nm thick gold layer was deposited onto the topside of the Py-SiNx platform *via* electron beam evaporation before deposition of PDMS.

The plasmonic bullseye structures and nanopore were milled into the Py-SiNx devices using focused ion-beam. The bullseye was milled using a Ga+ ion beam (FIB, Zeiss Auriga FEG SEM FIB, acceleration voltage = 20 kV, milling current = 5 pA). The milling times were adjusted to achieve the optimised bullseye geometry. To enhance visualisation, alignment markers were made surrounding the free standing membrane, allowing for easy location of the membrane in FIB mode. Alignment markers were deposited using the same Zeiss Auriga instrument, utilising SEM exposure in the presence of a gaseous precursor and an acceleration voltage of 20 kV.

Nanopore milling (ø – 10 - 80 nm) was carried out using a Helium ion beam (Carl Zeiss ORION NanoFab, beam current ~ 3 pA, aperture = 20 μm). The precise milling times (~20 - 30 s) were optimised through test milling, prior to nanopore fabrication. The overall bullseye/nanopore structure was imaged upon completion of the milling process. The devices were subsequently stored in a sealed container, and plasma cleaned prior to analysis.

***Plasmonic Heating Experiments:***

A custom built optical microscope was used to conduct the plasmonic heating experiments. The Py-SiNx devices were fixed to a fluidic cell, separating two solution filled compartments, each containing a non-polarizable Ag/AgCl electrode. The fluidic cell comprised of an upper and lower electrolyte reservoirs, each filled with aqueous KCl (100 mM) with 10 mM Tris and 1 mM EDTA (pH 8) buffer, separated by the Au/SiNx membrane. All the translocation experiments used 10 kbp DNA, and were diluted to a final concentration of 1 nM in the buffered solution and filtered using a 0.2 μm filter. The pores were heated using 532 nm, 632.8, and 685 nm wavelength light, the light sources were Nd:YAG, HeNe and diode lasers respectively. Each source was focused onto the nanopore entrance, using a diffraction limited spot (Ø ~ 3 µm). This fully illuminated the bullseye/nanopore structure with the Gaussian beam. Each variation in laser power was accompanied by analysis using multiple chronoamperometric traces (ranging from −0.3 to 0.3 V with a 0.025 V step and 1 s step duration), and monitoring of any translocation behaviour - using an A-M 2400 patch clamp amplifier, with 5 KHz Bessel filter and then sampled at 20 KHz with National Instruments NI USB-6251 DAQ and WinWCP 5.11 acquisition software. I-V plots were extracted from the chronoamperometric traces, with the slope yielding the nanopore conductance. The ionic current was monitored while varying the optical density of the excitation source.

***Numerical Heat Calculations:***

In the absence of phase transformations, the temperature distribution around optically stimulated surfaces can be described by the usual heat transfer equation:

(1)

where r and t are the coordinate and time, T(r,t) is the local temperature, and the material parameters ρ(r), c(r) and k(r) are the mass density, specific heat, and thermal conductivity, respectively. The solution of Equation (1) has a transient state, and after a characteristic time, it reaches its steady state [1-4] refs. Thermal processes in metals are fast, which means that a steady state is rapidly reached for typical incident powers and metal surface dimensions [5,6]. The function Qe(r,t) represents an energy (heat) source coming from light dissipation (EM losses) in the materials (see refs. [7,8] for more details):

In our case, the whole process of light absorption and subsequent heat transfer between the nanostructure and the surrounding medium has been modelled by means of finite element simulations. For easy implementation and reliability of the solution, we have chosen Comsol Multiphysics 4.3a (Comsol Inc., Burlington, MA), which provides state-of-the-art routines to solve partial differential equations (PDEs). In our simulations, we have assumed the EM losses from the light-matter interaction as the only heat source. We furthermore and unless otherwise stated made the adiabatic assumption, that is, we have assumed that the EM cycle time (≈femtoseconds) is short compared to the thermal time scale (≈nanoseconds). In order to take into account heat dissipation in our simulation area, we used a heat flux node across the outer boundaries, defined by the equation:

(2)

where h is the heat transfer coefficient of each medium, dependent on the geometry, material and the ambient flow conditions, Text is the external temperature (assumed to be the same as the initial temperature of the system), and T is the temperature of the system. The heat transfer coefficient h can often be estimated by dividing the thermal conductivity of the convection fluid by a length scale [9].

***Nanopore Estimates through Conductivity Calculations***

The electrical conductivity of the nanopore can be used to estimate approximate pore diameter. This incorporates the following assumptions; (i) the nanopore is uniform cylindrical hole transecting the free-standing membrane, (ii) the nanopore is the only source of resistance in the circuit, and (iii) the electrolyte solution is fixed at 100 mM KCl at a temperature of 20°C. The following equation (3) is used to estimate the cross-sectional area of the nanopore:

**A** = **G** × ***l*** / **σ** (3)

Where **A** is the cross-sectional area, **G** is the electrical conductance of the nanopore, ***l*** is the length of the nanopore, and **σ** is the conductivity of the electrolyte. The nanopore conductivity is experimentally measured at 0.54 nS, the length is 70 nm (total of the layered membrane; 50nm gold / 20 nm SiNx), and the electrolyte reference conductivity is 11.6159 mS/cm. This provides an estimated area, and nanopore diameter of 32.54 nm2, and 6.44 nm respectively.

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**Figure S1**. COMSOL modelling of the wavelength dependence of the (**A**) electric field enhancement, (**B**) relative absorption cross-section, and (**C**) relative temperature change (arbitrary units) for the Py-SiNx devices averaged over the whole nanopore – provided variation in the thickness of gold film present in the milled grooves of the bullseye rings (0-50 nm). Such that; 50 nm indicates that no gold has been removed (i.e. no bullseye), and 0 nm represents complete removal of the gold layer. For reference, the three laser lines are indicated on each plot (532 nm, 632.8 nm, and 685 nm). The simulated data is drawn from a 2D pseudo cross-sectional model of the bullseye system, and is an artificial cross-section which facilitates the comparison of the relative influence when varying the thickness of gold left in the bullseye rings. As a result, absolute values for the absorption cross-section, and temperature cannot be fully resolved – therefore relative values are provided. The 2D modelling also results in slight differences in the magnitude of electric filed enhancement (provided in Figure 3A).

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**Figure S2**. A line plot for simulated temperature change results for bullseye devices heated using a 5 mW beam at the given wavelength, and illumination width. The temperature data is taken from a 1D line that segments the diameter of the bullseye rings, and contacts the top surface of the gold layer. The steps observed in the central parts of the plot denote changes in heating behaviour caused by the presence of the rings of the bullseye structure – i.e. a displacement of 0 is at the centre of the nanopore, in-line with the top surface of the gold.

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**Figure S3**. Electrical noise analysis. (**Upper**) Shows the change in the amplitude of periodic electrical noise as the power of the 532 nm laser is increased. No power dependent change in noise frequency was observed. (**Lower**) Power spectrum density plots for the Py-SiNx devices, with no laser applied, and full power 632.8 nm and 532 nm lasers, at an applied potential of 450 mV. The broad frequency periodic noise can be clearly seen in the region between 700-2500 Hz.

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